United States
Environmental Protection
Agency

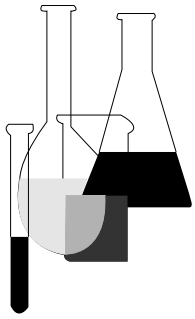
Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–96–139
April 1996

EPA

Ecological Effects Test Guidelines

OPPTS 850.2100 Avian Acute Oral Toxicity Test



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines’’ or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

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OPPTS 850.2100 Avian acute oral toxicity test.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline are 40 CFR 797.2175 Avian Acute Oral Toxicity Test and OPP 71–1 Avian Single-Dose LD50 Test (Pesticide Assessment Guidelines, Subdivision E—Hazard Evaluation; Wildlife and Aquatic Organisms) EPA report 540/09-82-024, 1982.

(b) **Purpose.** This guideline is designed to develop data on the acute oral toxicity to northern bobwhite and mallard of chemical substances and mixtures subject to acute environmental effects test regulations. The Agency will use these and other data to assess the acute hazard to birds.

(c) **Definitions.** The definitions in section 3 of the Toxic Substances Control Act (TSCA), section 3 of FIFRA, and 40 CFR Part 792—Good Laboratory Practice Standards apply to this test guideline. In addition, the following definitions apply to this guideline:

Acclimation Physiological or behavioral adaptation of test animals to environmental conditions and basal diet associated with the test procedure.

LD50 The empirically derived dose of the test substance that is expected to result in mortality of 50 percent of a population of birds which is treated with a single oral dose under the conditions of the test.

Test substance is the specific form of a chemical or mixture of chemicals that is used to develop the data.

Observation period is the portion of the test that begins after the test birds have been dosed and extends at least 14 days.

Hatch is eggs or birds that are the same age and that are derived from the same adult breeding population, where the adults are of the same strain and stock.

(d) **Test procedures**—(1) **Summary of test.** (i) After birds have been obtained, they are acclimated for at least 14 days. The dosage levels for the definitive test are established, possibly requiring a range-finding test to be conducted first. Test birds are randomly assigned to the various dosage levels and controls. Birds are weighed and the test substance is administered as a single oral dose either by gavage or capsule. Birds are closely monitored for 60 to 120 min after doses are given and then observed regularly for mortality or any signs of intoxication throughout the observation period. Birds are weighed and feed consumption is estimated at least weekly. The mortality pattern is examined and subjected to the appropriate statistical analysis to derive the LD50, confidence limits, and slope of the

dose-response line. The complete mortality pattern, along with signs of intoxication and necropsy data, should be reported.

(ii) A test is unacceptable if more than 10 percent of the control birds die during the test.

(2) **Range-finding test.** Unless the approximate toxicity of the test substance is known already, a range-finding test should be conducted to determine the dosage levels of the test substance to be used in the definitive test. Refer to paragraph (d)(3)(iv) of this guideline for details on dosage levels for definitive tests. Procedures for range-finding tests may vary, but generally, groups of a few birds are administered three to five widely-spaced doses. A series of 2, 20, 200, and 2,000 mg/kg-bw is suggested. If a test substance is expected to be of low toxicity, it may be useful to conduct a limit test at 2,000 mg/kg-bw first under paragraph (d)(3)(iv)(B) of this guideline. If mortality occurs at this level, then further range-finding at lower levels is suggested. The results of the range-finding test then may be used to establish the definitive test dosage levels.

(3) **Definitive test**—(i) **Administration of test substance.** (A) After acclimation under paragraph (e)(1)(i)(D) of this guideline, feed should be withheld from all test groups for a minimum of 15 h prior to administration of the test substance. Dosing by gavage is preferred—where gavage is not feasible, doses may be administered by gelatin capsule. Doses are to be based on the individual body weight (bw) of each bird. Body weights are typically determined at the time of dosing, but may be taken, especially for capsules, within 24 h prior to dosing. Dosing should be done in the early morning hours.

(B) If a carrier is used to administer the test substance, the preferred carrier is distilled or deionized water unless the test substance is known to hydrolyze readily. Other acceptable carriers include corn oil, propylene glycol, 1 percent carboxymethylcellulose, and gum acacia. Materials with known toxic or emetic properties should not be used. The dosing volume of test substance plus carrier in a test should be constant for all birds with respect to individual body weights and should not exceed 5 mL/kg-bw. For those unusual test substances that might require a larger dosing volume (e.g. liquids with low purity), a dosing volume up to 8 mL/kg-bw may be used; however, the test species should be bobwhite or else steps should be taken to ensure that mallards do not regurgitate the dose.

(ii) **Controls.** (A) A concurrent control is required during every test. Control birds should be from the same hatch as the test groups. Control and test birds should be kept under the same experimental conditions. The test procedures should be the same for control and treated birds, except that no test substance should be administered to the control birds. Control birds should receive a sham dose consisting of the same carrier or capsule as received by the test birds. The use of shared controls is acceptable for

concurrent tests as long as the same carrier or capsule is used for all the tests.

(B) A test is not acceptable if more than 10 percent of the control birds die during the test period.

(C) A concurrent positive control with a substance of known toxicity is not required. However, a quarterly or semiannual test with a laboratory standard (reference toxicant) is recommended as a means of detecting possible interlaboratory or temporal variation. A laboratory standard is also recommended when there is any significant change in food, housing, or source of birds.

(iii) **Number and sex of animals tested.** (A) In the definitive test, a minimum of 10 birds should be used for each dosage level of the test substance and for the control. Equal numbers of birds should be used for each dosage level.

(B) Birds at a dosage level may be divided into two pens of five birds each. If this is done, dividing the groups by sex is encouraged.

(iv) **Concentrations and dosage-mortality data.** (A) A minimum of five dosage levels of the test substance should be used in the definitive test. These levels should be spaced geometrically. The recommended spacing is for each dosage level to be at least 60 percent of the next higher level (less than 1.67 times the next lower level). Ideally, dosage levels should be spaced so that at least three levels result in mortality between, but not including, 0 percent and 100 percent; at least one level should kill more than 50 percent, and at least one level should kill less than 50 percent of the birds in a group. For some test substances, it may be necessary to use more than five dosage levels to achieve these results.

(B) For test substances expected to have relatively low toxicity, a limit test may be conducted at 2,000 mg/kg-bw. The LD50 may be reported as greater than 2,000 mg/kg-bw if 10 birds are dosed at 2,000 mg/kg-bw, if no mortality occurs, and if test procedures, number of controls, and duration are the same, except for the number of dosage levels, as in the definitive test. Signs of intoxication, if any, should be reported. No further testing is required at lower dosage levels.

(v) **Duration of test.** The definitive test consists of the administration of the test substance followed by an observation period of at least 14 days. If mortality occurs during the last 3 days of the 14–day period, or if signs of intoxication are not clearly in remission, or if the test substance is expected to have delayed effects, then the observation period should be extended to at least 21 days or until mortality or signs of intoxication are not observed for 72 hours.

(vi) **Observations.** (A) Birds should be monitored closely for the first 60 to 120 min after dosing. Any regurgitation should be noted and reported. Additional observations of test birds should be made, at a minimum, 3 times on the day of dosing and at least daily throughout the remainder of the test period. Where feasible, twice daily observations are recommended.

(B) Throughout the test period, all signs of intoxication, other abnormal behavior, and mortality should be recorded and reported by dosage level, by sex, and by day. Signs of intoxication are those behaviors apparently due to the test chemical and may include a wide array of behaviors, such as labored respiration, leg weakness, hemorrhage, convulsions, ruffled feathers, etc. All signs of intoxication and any other abnormal behavior, such as excessive aggression, toe-picking, etc. that may or may not be attributed to the test substance should be reported. Among survivors, remission of signs of intoxication and cessation of abnormal behavior should be recorded by dosage level and by day. An estimate of the number of birds exhibiting such signs should be recorded for each dosage level.

(C) Individual body weights of birds should be recorded and reported for control and treated birds at the time of calculating the dosage to be administered and weekly thereafter until the test is concluded. An extra weighing the third day after dosing may provide useful information, especially on anorexia. Body weights of birds a week prior to dosing are not required, but would provide valuable base-line data. Feed consumption should be recorded at least weekly throughout the test; valuable additional information can be obtained by monitoring food consumption daily, especially for the first few days following dosing.

(D) Gross pathology examinations should be conducted on at least two or three birds dying at each dosage level and on all control birds that die. Gross pathological examinations of survivors are optional, but may provide valuable information, especialy for lesions associated with sublethal effects.

(4) **Analytical measurements—**(i) **Statistical analysis.** (A) The data should be analyzed, preferably by graphical or computational methods of probit analysis. (See paragraphs (g)(4) and (5) of this guideline.) The LD50 value, 95 percent confidence limits, and slope of the transformed dose-response curve should be determined for mortality at the end of test. A test for heterogeneity of the data (e.g. X^2 test) should be conducted. Other standard statistical methods are acceptable if they provide the slope of the dose-response line as well as the LD50 value.

(B) All methods used for statistical analysis should be described completely.

(ii) **Analysis of basal diet.** (A) A proximate analysis of the basal diet should be included in the test report. The analysis should include per-

centages by weight of protein, fat, fiber, ash, calcium, and phosphorus. In addition to these analyzed components, a list of expected amounts of vitamins, minerals, or other supplements also should be reported. Most commercial feed companies provide both the analysis and the list of supplements on the label.

(B) A contaminants analysis of the feed should be conducted periodically for heavy metals (e.g. arsenic, cadmium, lead, mercury, and selenium) and persistent pesticides, especially chlorinated insecticides. A broader pesticide screen to include, for example, diazinon, methyl parathion, and malathion may be useful.

(e) **Test conditions—**(1) **Test species—**(i) **Selection.** (A) Northern bobwhite, *Colinus virginianus* (L.), and mallard, *Anas platyrhynchos* L., are the test species. Birds may be reared in the laboratory or purchased from a breeder. All control and treatment birds used in a test should be from the same source and breeding population. Birds should be obtained only from sources whose colonies have known breeding histories. Birds should be phenotypically indistinguishable (except for size) from wild stock. It is recommended that birds be obtained from flocks that have been outbred periodically in order to maintain a genetic composition that approximates the natural heterogeneity of the species.

(B) Birds used in the test should be in apparent good health. Deformed, abnormal, sick, or injured birds should not be used. Birds should not be used for a test if more than 5 percent of the total test population die during the 14–day acclimation period. Birds purchased from a breeder should be certified as disease-free or as bred from disease-free stocks. Birds should not have been selected in any way for genetic resistance to toxic substances. Birds should not have been used in a previous test, either in a treatment or control group.

(C) Test birds should be young adults, not yet mated, at least 16 weeks old at the time of dosing. A less preferred alternative is for the use of first-year birds that may have been mated, as long as the birds are brought completely out of production through reduced light cycles. All birds used in a test should be the same age ± 1 week. It is recommended that weights be at least 180 g for bobwhite and 900 g for mallard. More consistent responses may be attainable if the range of body weights is no greater ± 10 percent of the mean body weight for the test population. The age should be recorded and reported.

(D) Test birds should be acclimated to test facilities and basal diet for a minimum of 14 days. Acclimation to test pens should be in the actual pens used in the test. Birds used in the test should be assigned randomly to treatment and control pens, except that assignment may be made to result in only one sex per pen if replicate pens are used for each dosage

level, under paragraph (d)(4)(iii) of this guideline. Randomization should be done at the initiation of the acclimation period.

(E) During holding, acclimation, and testing, birds should be shielded from excessive noise, activity, or other disturbance. Birds should be handled only as much as is necessary to conform to test procedures.

(ii) **Diet.** (A) A standard commercial game bird (for bobwhite) or duck (for mallard) feed or the nutritional equivalent, should be used as the diet. Feed should not be used past its normal shelf life. Antibiotics or other medication should not be used in the diet during the acclimation period or the test. It may not be possible to obtain feed that is completely free of pesticides, heavy metals, and other contaminants; however, diets should be analyzed periodically, under paragraph (d)(6)(ii)(B) of this guideline, and selected to be as free from contaminants as possible. Extra precautions should be taken when fish meal or oil is a major ingredient, since fish are often contaminated with high levels of chlorinated hydrocarbons.

(B) Clean water should be available ad libitum. Only clean, unmedicated water should be offered during the acclimation and testing periods. Water bottles or automatic watering devices are recommended. If water pans or bowls are used, water should be changed at least once a day.

(2) **Facilities.** (i) Tests should be conducted indoors with birds being maintained in commercial breeder or holding pens or pens of similar construction. Pens should be constructed of galvanized metal, stainless steel, or perfluorocarbon plastics. Materials that are toxic, likely to influence toxicity, or sorb test substances should not be used. Wire mesh should be used for floors and external walls; solid sheeting should be used for common walls and ceilings. Wire mesh for floors should be fine enough so as to not interfere with the normal movement of birds yet coarse enough to allow fecal material to fall through. Pens should have a floor area of at least 500 cm^2 per bird (approximately 75 in^2) for bobwhite and 1,000 cm^2 per bird (approximately 150 in^2) for mallards and should be at least 24 cm (approximately 9.5 in) high for bobwhite and 32 cm (approximately 12.5 in) high for mallard. Between tests pens should be disassembled (if feasible) and should be cleaned thoroughly. Steam cleaning of cages is recommended. Cages may be hosed, brushed thoroughly and hosed again, as an alternative method. The use of detergents or bleach is acceptable, but other chemical disinfectants such as quaternary ammonium compounds should not be used. When necessary to control disease vectors, hot or cold sterilization techniques are recommended, as long as such techniques will not leave chemical residues on the cages. For cold sterilization, ethylene oxide is recommended. Pens should not be cleaned during a test.

(ii) Testing is done indoors to control lighting and other environmental variables. Temperatures for adult birds should be maintained at normal indoor temperatures, preferably between 15 °C and 27 °C (60 to 80 °F). Ventilation should be sufficient to supply 10 to 15 air changes per hour. The test room should be maintained at a relative humidity of 45 to 70 percent. Higher humidities are appropriate for waterfowl. A photoperiod of 8 h light and 16 h dark is recommended in order to prevent birds from coming into reproductive condition. Lighting may be either incandescent of fluorescent. Pens and lights should be positioned so that all pens will receive approximately equal illumination.

(f) **Reporting.** (1) The report should include, but not necessarily be limited to, the following information:

(i) Name and address of the facility performing the study and the dates on which the study was initiated and was completed, terminated, or discontinued.

(ii) Objectives and procedures stated in the approved protocol, including any changes in the original protocol.

(iii) Statistical methods employed for analyzing the data.

(iv) The test and, if used, control substances identified by name, Chemical Abstracts Service (CAS) number or code number, source, lot or batch number, strength, purity, and composition or other appropriate characteristics.

(v) Stability of the test and, if used, control substances under the conditions of administration.

(vi) A description of the methods used, including:

(A) Description of housing conditions, including type, size, and material of pens, and the approximate test room temperature, humidity, ventilation rate, photoperiod, and lighting intensity.

(B) Detailed description of feed, including source, supplements (if used), and approximate analysis.

(C) Acclimation procedures and methods of assigning birds to test pens, and test pens to dose-levels.

(D) Frequency, duration, and methods of observations.

(vii) A description of the test system used, including the scientific name of the test species, number used, sex and reproductive history and condition, age (in weeks) at the beginning of the test, source, and procedures used for identification. Individual body weights (or means, extremes, and an estimate of variance) should be reported for the beginning of the test and weekly thereafter.

(viii) A description of the dosages, numbers of birds and replicates per dose, method and time of administration. The reported results should include:

(A) The results of range-finding tests, if conducted.

(B) For the definitive test, a description of signs of intoxication and other abnormal behavior, including time of onset, duration, severity (including death), and number affected at each dose level and control each day of the test.

(C) Feed consumption per pen at least weekly or as often as measured, if more frequently than weekly, along with an estimate of wastage.

(D) The results of gross pathological examinations.

(ix) A description of all circumstances that may have affected the quality or integrity of the data.

(x) The name of the sponsor, study director, principal investigator, names of other scientists or professionals, and the names of all supervisory personnel involved in the study.

(xi) A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis. Results of the analysis of data should include the calculated LD50 value, 95 percent confidence limits, slope of the transformed dose-response line, and the results of a goodness-of-fit test (e.g. X^2 test).

(xii) The signed and dated reports of each of the individual scientists or other professionals involved in the study, including each person who, at the request or direction of the testing facility or sponsor, conducted an analysis or evaluation of data or specimens from the study after data generation was completed.

(xiii) The locations where all specimens, raw data, and the final report are stored.

(xiv) The statement prepared and signed by the quality assurance unit.

(g) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Daum, R.J. Revision of two computer programs for probit analysis. *Bulletin of the Entomological Society of America* 16:10–15 (1970).

(2) Daum, R.J. and W. Killcreas. Two computer programs for probit analysis. *Bulletin of the Entomological Society of America* 12:365–369 (1966).

(3) Finney, D.J. *Probit Analysis* 3rd ed., Cambridge: London and New York (1971).

(4) Litchfield, J.T., Jr. and F. Wilcoxon. A simplified method of evaluating dose-effect experiments. *Journal of Pharmacological Experimental Therapy* 96:99–133 (1949).

(5) Stephan, C.E. *Methods for calculating an LC50.* Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, American Society for Testing and Materials, Philadelphia, PA (1977) pp 65–84.